Artificial Fertilization Among Yellowfin and Gulf Menhaden (*Brevoortia*) and Their Hybrid

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ABSTRACT

Eggs of yellowfin menhaden, *Brevoortia smithi*, were artificially fertilized with sperm from yellowfin menhaden, Gulf menhaden (*B. patronus*), and naturally occurring hybrid menhaden, and reared through the larval yolksac stage at sea under controlled temperature and salinity. Hatching occurred after 38 hours at 21 C. The last larva died 7 days after hatching. Specimens of embryos and larvae were preserved during development and later photographed. Survival of the cross-fertilized eggs equalled that of the intraspecific fertilizations.

INTRODUCTION

Despite extensive investigation of the Nation's valuable menhaden resources (*Brevoortia*: Clupeidae), much is still unknown about the early life history of the various menhaden species. One objective of Bureau of Commercial Fisheries research is to determine more precisely where, when, and how intensively menhaden spawn each year. Studies of the distribution and abundance of the menhaden's planktonic eggs and larvae should yield this knowledge, but these early stages have not been described for all species and their positive identification in biological collections remains a vexing problem in some areas. Early descriptions of Atlantic menhaden (*B. tyrannus*) eggs and yolksac larvae were based on material found in plankton collections. References to these descriptions were listed by Reintjes (1962), who first described eggs and yolksac larvae obtained artificially from the yellowfin menhaden, *B. smithi*. Descriptions are lacking for eggs and larvae of the Gulf menhaden, *B. patronus*, and the finescale menhaden, *B. gunteri*, the former species being of great commercial value in the Gulf of Mexico.

Adding to the difficulty of identifying the planktonic stages of Atlantic, Gulf, and finescale menhaden without the aid of descriptions based on material of known parentage is the problem of identifying the eggs and larvae of menhaden produced by hybridization. Unpublished data collected during two years of exploratory fishing by the Bureau revealed that hybrids make up a substantial portion of the menhaden population along the west coast of Florida between Tampa Bay and Florida Bay. Because many of our plankton collections have been made in areas where hybridization is likely, several questions needed investigation: (1) to what extent are the gametes of sympatric menhaden species interfertile, (2) are the developing hybrid embryos and larvae distinguishable from those produced through intraspecific fertilizations, and (3) are the gametes of naturally occurring hybrids viable?

This paper presents the results of artificially fertilizing eggs of yellowfin menhaden with sperm from Gulf menhaden, yellowfin menhaden, and naturally occurring hybrids of these two species. Mature eggs of the Gulf menhaden or of the hybrids were not available during the experiments, thus reciprocal crosses could not be attempted.

COLLECTION OF RIPE ADULTS

Identification of planktonic fish eggs and larvae entails their comparison with developmental series of eggs and larvae of known parentage. Perhaps the best way to obtain such a series is to fertilize the eggs artificially from mature fish of unquestionable identity and rear the resulting larvae. Monthly cruises of the Bureau's exploratory fishing vessel R/V *George M. Bowers* in the eastern Gulf of Mexico, during the spawning season (November–March), offered opportunities to obtain running-ripe fish.

Monofilament gill nets were used to catch menhaden inside the 20-fathom line between Panama City and Cape Sable, Florida. The nets were set, during daylight, at various depths for one hour at each station. Quick retrieval of the nets yielded fresh specimens with viable eggs and sperm.

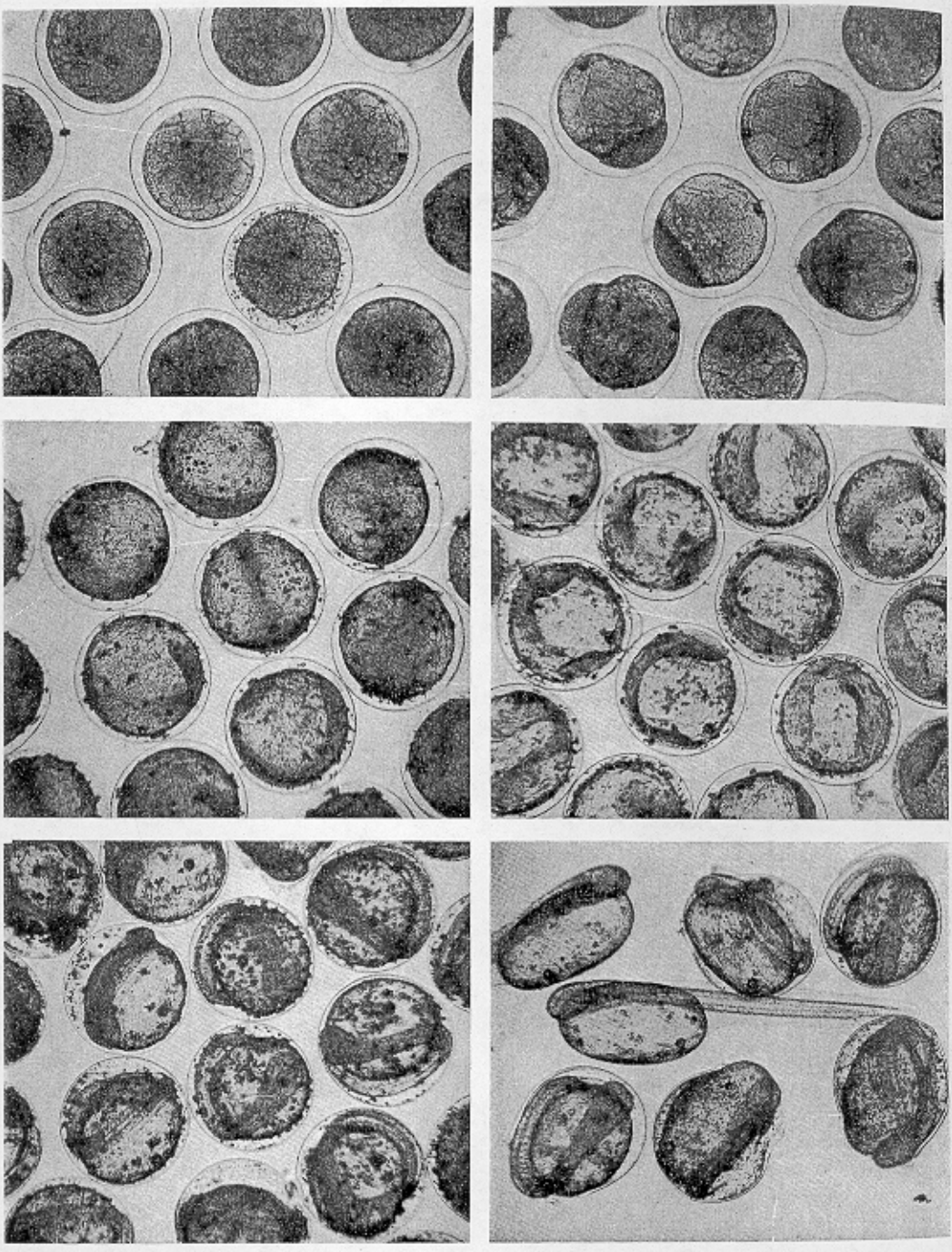

FIGURE 1.—Eggs of *B. smithi* fertilized with *B. patronus* sperm. Left to right, top to bottom: perivitelline space developed (1 hr after fertilization); high blastula (6 hr); early neurula (15 hr); late neurula with head region developing opposite oil droplet (20 hr); late embryo (36 hr); hatching (38.5 hr). The larva in bottom right frame is 3.2 mm long.

TABLE 1.—*Comparison of the seven menhaden used in fertilization trials*

Fertilization group	Species	Sex	Fork length (mm)	Modified predorsal scales	Oblique rows of scales along side	Opercular striae	Margin of pelvic fin	Secondary spots	Frontal groove
I	*B. smithi*	F	224	48	72	weak	oblique	none	none
	B. smithi	M	217	44	73	weak	oblique	none	none
	Hybrid	M	236	41	57	weak	square	none	short
	B. patronus	M	208	32	44	strong	convex	present	long
II	*B. smithi*	F	186	39	68	weak	oblique	none	none
	B. smithi	M	215	46	70	weak	oblique	none	none
	B. patronus	M	226	31	46	strong	convex	none	long

No running-ripe females were caught on six cruises before March 1966, although some females appeared nearly ripe and many males had free-flowing milt. Finally, on the afternoon of 17 March, we collected one ripe female yellowfin menhaden, along with 265 other menhaden 10 miles north of Naples, Florida, in 3 fathoms. Of these fish, 96% were males, most of which were ripe. The predominance of apparent hybrids in this catch (74%), all of which were males, contributed to the unbalanced sex ratio. Seventeen percent of the total catch were Gulf menhaden and 9% were yellowfin menhaden. At this location the water temperature was slightly over 20 C and the salinity was 34‰. The only other ripe female, a smaller yellowfin menhaden, was caught the following afternoon along with 73 other menhaden 1 mile south of Sanibel Island in 2 fathoms of water. Of this catch 80% were males (nearly all ripe); the species composition was 41% hybrids, 5% Gulf menhaden, and 54% yellowfin menhaden. Water temperature and salinity were 23 C and 33‰ respectively.

Each specimen used in the fertilization trials was identified with the aid of descriptions by Hildebrand (1963). The more diagnostic meristic and qualitative characters, including that of the length of the groove above the frontal bones (Dahlberg, 1966), are compared in Table 1.

FERTILIZATION AND REARING

The eggs were fertilized and incubated at sea while the research vessel continued its exploratory cruise. Eggs from the first yellowfin menhaden (Fertilization Group I) were stripped into three dry glass bowls. Several drops of sperm from a male of the same species were mixed with the egg mass in the first bowl. The eggs in the second bowl received sperm from a hybrid, and the eggs in the third bowl were fertilized with sperm from a Gulf menhaden. After 1 to 2 minutes, about 100 ml of filtered sea water were added to each bowl. The eggs, sperm, and water were agitated for the next 5 minutes, and then poured into a 1-liter jar two-thirds full of sea water. The fertilized eggs floated to the surface within 30 minutes and were decanted into additional jars containing sea water. The remaining unfertilized eggs and ovarian tissue were discarded. At this stage, each culture jar contained about 1,500 developing eggs. Eggs from the second ripe female (Fertilization Group II) were stripped into two bowls and fertilized in a similar manner by sperm from a yellowfin menhaden and a Gulf menhaden. Space limitations in the incubator prevented culturing a yellowfin-hybrid cross in this second group. A portable thermostatically controlled refrigerator–heater held the incubation water bath for the rearing jars at 20.5 ± 1.0 C, which corresponded to the temperature of the nearshore Gulf of Mexico. A small air pump kept the water aerated and in motion. When the sea was rough, the jars were removed from the constant-temperature bath, placed in a rack to reduce spillage, and held at room temperature of about 21 C. Part of the water in each jar was replaced twice daily with filtered sea water.

Eggs were sampled and preserved at 1- to 4-hour intervals until hatching. Specimens of larvae were removed every 12 to 24 hours thereafter until the last larva died. Upon absorption of the yolksac, the larvae were fed small plankters and small amounts of finely chopped egg yolk in an attempt to keep them alive. We had no evidence that the larvae fed, however, and all soon died.

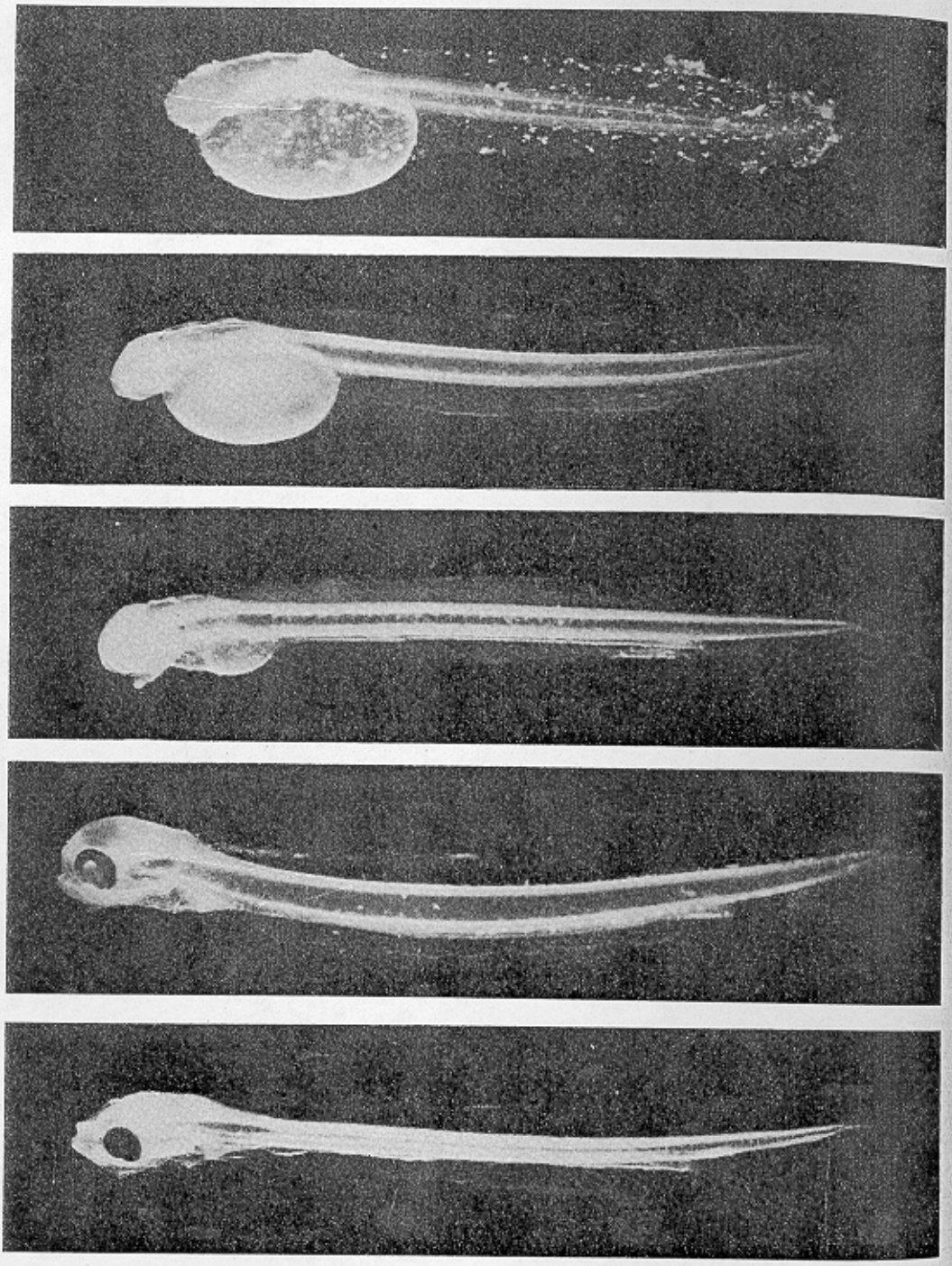

FIGURE 2.—Larvae produced from *B. smithi* female *B. patronus* male. Top to bottom: 3.6 mm (6 hr after hatching); 3.9 mm (26 hr); 4.2 mm (58 hr); 4.5 mm (82 hr); 4.3 mm (130 hr). The eyes became pigmented 70 hours after hatching and the yolk was completely absorbed after 80 hours. The length and thickness of the larvae decreased as is shown in the last frame, this decrease reflecting their failure to feed.

RESULTS OF FERTILIZATIONS

Cross-fertilization of the yellowfin menhaden eggs using sperm from hybrid and Gulf menhaden males produced embryos and larvae visually indistinguishable from those of intraspecific fertilizations. Further, the percentage of eggs that hatched was similar for all fertilizations. Figures 1 and 2 are composite photographs of some stages of one preserved series of yellowfin menhaden eggs fertilized with Gulf menhaden sperm. Direct comparison of these eggs and larvae with preserved eggs and larvae of yellowfin menhaden obtained by Reintjes (1962) from the Indian River in eastern Florida showed that his descriptions of embryo and yolksac larvae development were applicable with the exception of the egg diameter and the ratio of perivitelline space to yolk diameter. Diameters of the fertilized eggs, measured after preservation in 4% neutralized formalin, ranged from 1.05 to 1.18 mm. Their average yolk diameter was 0.98 mm. In comparison, the diameter of eggs artificially fertilized by Reintjes (1962) ranged from 1.15 to 1.30 mm, their average yolk diameter being 0.86 mm. The individual fish's diet, maturity, and spawning history possibly account for the differences in yolk diameter. The smaller amount of perivitelline fluid in the eggs from the Gulf of Mexico may be related to the somewhat higher salinity in which the eggs were fertilized (33–34‰ in the Gulf of Mexico, 26–27‰ in the Atlantic).

Hatching time for the five lots of fertilized eggs reared at 19.5 to 21.5 C was 38 to 39 hours. Hatching time is defined as the time for half of each batch of fertile eggs to hatch. Eggs obtained in similar fashion from yellowfin menhaden along the east Florida coast hatched in 46 hours when held at 18 C, 34 hours at 21 C, and 26 hours at 26 C (John W. Reintjes, personal communication).

SIGNIFICANCE OF FINDINGS

The material and information described in this paper have several practical applications. The developmental series will aid in the identification of planktonic menhaden eggs and larvae, and use of the data on rate of egg development to assign ages to menhaden eggs in biological collections can assist in more accurate location of spawning areas.

The artificial cross-fertilization of yellowfin menhaden eggs with Gulf menhaden sperm showed that the gametes of the species are compatible. The eggs had a hatching potential and the larvae had vigor equal to those of eggs and larvae produced from intraspecific fertilizations of yellowfin menhaden. Since the mature adults of each species occur together during their breeding seasons, natural cross-fertilization and the production of hybrids also are likely. Fish that appeared to be intermediate between the two species were abundant in our catches. The ability of apparent hybrid males to fertilize yellowfin menhaden eggs suggests also that backcrossing is possible. As we observed and as was noted by Dahlberg (1966), less than 1% of all menhaden hybrids were females. The lack of hybrid females would serve as an intrinsic barrier to the production of a self-sustaining population of hybrids and ensure the integrity of the two species.

LITERATURE CITED

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